Comments Regarding the National Toxicology Program Vision for the 21st Century

Subcommittee of the National Toxicology Program Board of Scientific Counselors

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Introduction:

The National Toxicology Program was established in 1978. As we move into a new century, it is an appropriate time to reevaluate the direction of the NTP. Its members have put forward a vision for the 21st century as follows: To move toxicology from a predominantly observational science at the level of disease-specific models to a predominantly predictive science focused upon a broad inclusion of target-specific, mechanism-based, biological observations. They have enlisted several outside groups of individuals to review this vision, including the National Toxicology Program Board of Scientific Counselors. A subcommittee was formed by the Board of Scientific Counselors to comment on the vision and to address some of the specific questions accompanying the description of the vision and roadmap for the NTP.

This subcommittee has met together on two occasions and by conference call and individual calls on other occasions. In addition, comments to the subcommittee were made at a forum in Baltimore following the Society of Toxicology annual meeting on Thursday, March 25, 2004, at the Hyatt Hotel, from 1:00-5:00 p.m. Presentations were made by Dr. Linda Birnbaum (U.S. EPA), Dr. James Bus (Dow Chemical Company), Dr. David Eaton (University of Washington), Dr. William Greenlee (CIIT Centers for Health Research), Dr. Carol Henry (American Chemistry Counsel), Dr. Robert Kavlock (U.S. EPA), and Dr. James Popp (Purdue Pharma L.L.P.). After consideration of the comments by these presenters and additional discussion between members of the subcommittee, the following report is presented to the full Board of Scientific Counselors for its consideration for comment to the National Toxicology Program.

Overview:

In general, all commenters and members of the subcommittee are strongly supportive of the emphasis on the vision of the NTP for the 21st century for a transition from an observational science to a predictive science focused on target-specific, mechanism-based, and biological observations, with a strong emphasis on mechanism. However, all members of the subcommittee realize that this process needs to be accomplished in an orderly, validated procedure so that the current strengths of the NTP for testing and identifying potential toxicologic hazards for humans are not lost. Given the enormous number of chemicals that require evaluation, the current testing paradigm is not sustainable. Incorporation of more mechanistic and biologic information in combination with newer technologies is required to address these critical issues. It is well recognized that the NTP is critical for toxicological testing and represents a national and international resource for those involved in various aspects of risk assessment of a wide variety of chemicals for multiple purposes. Placing an emphasis on providing data that can be used for rational extrapolation from model systems to humans and incorporating dose and species differences and similarities are essential. This will require a team approach involving multiple disciplines to address these issues as well as extensive developments in, and the use of, bioinformatics. Partnership with other agencies and entities will be necessary to address many of these issues. Risk communication will be an increasingly important component of the process.

Comments to the specific questions posed to the subcommittee follow:

1. **What scientific information should the NTP be producing and what technical capabilities should the NTP have by 2008? By 2013?**

The mission of the NTP is to provide its sponsors and stakeholders with information on the potential hazards of chemical and physical agents that serves as the basis for assessing the risk of these agents to cause diseases in humans. Advances in technology and in the scientific understanding of disease etiology provide NTP with an unprecedented opportunity to make its output even more relevant to its mission. These advances should make it possible for the NTP to not only evaluate more chemical and physical agents, but to test smarter, using study designs with endpoints more directly related to specific human disease states, testing protocols developed to meet specific risk assessment needs, and testing strategies focused on the most likely manifestations of toxicity to be produced by the agent under investigation.

The NTP generates toxicity information using a variety of methods, but mostly by animal studies that are designed as models for predicting the outcome in humans of exposures of various durations (acute, subchronic, chronic) or during particular life stages (e.g., developmental toxicity assessments) or by assessing particular biological functions (e.g., reproductive toxicity assessments). These tests have served the purpose of identifying hazards and have provided the basis for risk assessments; however, most of the assays are time-consuming, labor-intensive and expensive. The number of chemicals that can be evaluated, and the number of tests that can be run, is limited by the magnitude of the resources needed to conduct them. As these resources become scarcer, it seems obvious that NTP's current screening and testing paradigm will no longer be sustainable.

The assay systems that are now used are apical; that is, they detect the ultimate manifestation(s) of toxicity, regardless of the modes of action by which these manifestations are produced. The apical test design has two invaluable intrinsic properties: first, the response that is measured is a sensor for effects emanating from the interaction of an insult with any of a potentially large number of biochemical or cellular targets; and secondly, the adverse response in the animal model (e.g., carcinogenic response) can be directly extrapolated to humans because it is thought to be predictive of a response in humans. Conversely, the very nature of the apical test makes it a black box; it is generally impossible to determine how a particular effect came about unless mechanistically linked measurements are added to the study.

The NTP is viewed by many in the regulatory arena (both government and industry) as the focal point for setting standards for toxicological testing. With the advent of additional scientific insight and new technologies, the NTP now has developed a vision of moving to a more mechanism-based approach. Although the Board strongly supports this direction, as it did in its last review nearly ten years ago, the Board cautions the NTP to do this in an orderly fashion. Tests should not be abandoned until it has been adequately demonstrated that newer approaches are inclusive and reliable enough to provide a sufficient assurance of lack of a hazard. This will require proper validation of these new tests. The issue of what standard to use for validation

remains contentious, but the Board strongly urges the NTP to focus its efforts on developing techniques for determining effects in humans, not effects in rodents, other species, or cellular test systems. Mechanistic applications require an understanding of the results of screening tests to extrapolate to humans at the levels to which they are exposed. The number of mechanisms that might be evaluated at any point in time will depend upon the completeness of understanding of the biologic processing of a chemical in humans. Disease-specific observations, i.e., bioassays and human epidemiology, will likely remain necessary for sound regulatory decisions for both scientific and political reasons.

To implement the vision, assays will initially need to be added to the classical NTP bioassays to expand the classical studies into predictive sciences. Continuing the animal bioassays for at least a period of time will allow validation of the new technologies. NTP must continue to be mindful of the balance in emphasis between testing, methods development and validation in this process. A key principle in expanding the classical bioassays will be to include assays that relate to developmental toxicology, cardiovascular toxicology, neurotoxicology, immunotoxicology and other toxicities that may be relevant to the compounds under test. It is important that animals be used for multiple endpoints in implementing this strategy.

The scientific concern is that insufficient information may lead to a false conclusion of lack of a hazard. The standard should not be perfection, but any battery of mechanistic-based tests should have a reasonably high probability of providing information that leads to correct judgments. There is also the opposite concern, i.e., that a comprehensive battery of short-term tests will result in too many false positive responses, which would have an economic impact and direct scarce health resources into unproductive areas. The political challenge is to obtain agreement from all important stakeholders regarding conclusions based entirely on mechanistic data. Certainly the current climate regarding interpretation of toxicologic results suggests that a small battery of mechanistic tests is unlikely to be persuasive to the stakeholder community at large. This will require research and the generation of data to support changes.

The NTP should remain the primary government institution devoted to evaluating hazards. This will require an assessment of the current neoplastic, neurologic, immunologic, reproductive, developmental, and endocrine outcomes and others. Evaluations that have more of an emphasis on function and/or behavior may ultimately be as important in predicting the adverse effects of environmental agents, as are more traditional evaluations of morphology. Some emphasis should be placed on the development of models for human disease states that have mainly functional manifestations. The extension over the past few years to include outcomes other than cancer needs to continue.

Animal and cellular models to evaluate the additional endpoints discussed above are needed. Efforts should also be undertaken to expand the capacity to evaluate intermediate endpoints and biologic prognosis markers instead of final disease outcomes. DNA, RNA, and protein arrays are just beginning to provide new insights into processes and disease characterization. The NTP should plan to incorporate these techniques into future activities as their usefulness is demonstrated and tests are validated.

It is essential for the NTP to expand its capabilities in mechanistic evaluations. The mechanistic focus seems especially valuable for identifying candidate chemicals for chronic

bioassays and for understanding the relevance of animal findings to humans. A core set of toxicologic responses should be developed to help prioritize agents for in-depth study. In developing this core set, the NTP should perform validation studies of established toxicants and nontoxicants to determine the false positive and false negative rates. This should be carried out before the core set is put into extensive use.

One of the principal uses of information derived from NTP studies is in risk assessment. Risk assessment requires both qualitative information on hazard posed by chemical exposure and quantitative information on the chemical's toxic potency (i.e., information on dose-response relationships). Historically the emphasis of the NTP has been on the qualitative aspects of identifying chemicals that may pose a hazard for reproductive effects, cancer, etc.; however, regulatory agencies and others have nonetheless relied upon NTP studies to develop the dose-response relationships needed to conduct quantitative risk assessments. As the NTP moves forward, it needs to acknowledge and accept its role in providing dose-response information of critical value to its stakeholders. This has important ramifications for both conventional and predictive, mechanism-based approaches to developing toxicity information.

At the most basic level, attention to needs for dose-response information can affect the number of doses chosen for testing and their spacing. The Board is mindful that the costs of studies escalate as the number of doses included increases, thus the value of information gained by expanding the number of doses in a particular study must be carefully weighed. However, as a general principle, studies should be designed to derive, as efficiently as possible, information that serves both qualitative and quantitative interests. On a more advanced level, there needs to be a greater consideration that toxicity is a function not only of dose, but also of time. This has implications not only in terms of windows of vulnerability to some chemicals, but also in determining how magnitude and duration of exposure together affect outcome. The NTP should be a leader in advancing an understanding of the interrelationships of dose and time in toxicity.

As the NTP moves toward the use of mechanism-based approaches for evaluating the toxic potential of chemicals, the need for dose-response information cannot be forgotten. However, even if this approach is shown to be highly reliable from a qualitative standpoint (i.e., few false negatives or false positives), its regulatory value is limited unless there is accompanying information on toxic potency. It will be of critical importance to distinguish between adaptive effects and adverse effects, particularly at low doses.

As the NTP looks to the future, it should develop both short-term and long-term goals. Short-term goals should be essentially refinements to current approaches to add value to existing types of studies. Suggestions for refinements include:

1) further incorporation of mechanistic experiments as part of NTP studies. The Board notes, and strongly supports, recent efforts by the NTP to include mechanistic experiments to aid in interpreting the human health relevance of its animal model studies. These efforts should be continued and expanded.
2) acquisition of "omic" information during testing, to develop a foundation for use of these technologies to predict toxicity;
3) for selected chemicals, adding experiments evaluating effects of dosing during potentially sensitive periods (e.g., perinatal exposure, exposure around puberty for

agents with hormonal effects); and

4) for selected chemicals, adding stop studies to examine the influence of dose duration on response.

Long-term goals may be directed toward replacing empirical tests of toxicity with faster, less expensive, predictive tests. The ability to move toward such tests relies on the concept that there exists a finite and manageable set of biological events that can serve as reliable predictors of toxicity. To build the foundation for this approach to toxicity evaluation, the NTP should lead efforts to identify and catalog these events. Some of the information for this effort could come from the mechanistic and "omic" additions to NTP studies, but the NTP does not have to be the sole, or even primary, contributor of data.

2. **How do you envision that the refinement/replacement of classical toxicological studies with mechanism-based assays will impact on the evaluation of public health hazards?**

Although knowledge of the mechanisms by which toxicants act is still far from exhaustive, it is extensive. There is enough information on the targets of toxicity that it is possible for the NTP to begin to assess chemicals in a mechanistic context.

There is an increasing understanding that different disease states may have a similar root cause at the molecular level, with the ultimate manifestation of disease dependent on factors such as the life stage at which the exposure occurred or the target tissue. *For example*, repeated exposure to estrogens during prenatal or perinatal development leads to persistent organizational effects on the reproductive system, whereas exposures during adulthood can lead to altered reproductive function. Furthermore, in humans, treatment with pharmacological levels of estrogens (unopposed by progestogens) can cause endometrial cancer and may also enhance the growth of breast cancer. Foreknowledge that a chemical to be tested by the NTP has potent estrogenic properties could lead to a more targeted testing program that focuses on the life stages and endpoints most likely to be susceptible. In this particular example, it could also lead to the selection of a non-standard model for evaluating carcinogenic potential, one that is more highly concordant with human carcinogenic responses. Likewise, screening for clinically significant immunosuppressive activity might, in itself, be predictive of carcinogenic activity in humans abrogating the need to conduct a two-year bioassay.

In a similar fashion, scientific insights into the mechanisms of carcinogenesis for several tissues have developed to the level that a more mechanism-oriented, short-term approach could be developed to provide more information to address issues of human relevance and dose-response relationships.

Given expanding knowledge of the underlying mechanisms of diseases (particularly toxicant-induced diseases), it seems clear that the NTP should focus on translating this basic information into pragmatic approaches to expand its ability to better predict the potential human toxicity of a greater number of chemical and physical agents. The key visionary elements for NTP over the next five years should include an emphasis on establishing links between early molecular events and ultimate manifestations of toxicity. This level of understanding could

facilitate the design of mechanism-based, relatively high-throughput screening methods to identify the most potent toxicants and to provide sufficient information to tailor the definitive testing of these toxicants so that the most likely targets/disease susceptibilities are the point of focus. Such information could also lead to an understanding of the quantitative relationships between the proximate interactions between toxicant and endogenous receptors at a molecular level, and the frankly adverse effects that occur at higher levels of biological organization. This information would also provide a knowledge base for a more routine biologically based dose-response modeling effort and help in the design of more predictive models, particularly for those prevalent human diseases for which, at least, a portion of the etiology is attributable to the environment.

Over the next 10 years, the vision should extend into developing a framework for both incorporating this information into a pragmatic testing scheme and assisting the recipients of NTP's output (particularly those involved in chemical regulation), in optimizing their risk assessment practices to take maximal advantage of the new types of data being generated.

Genomic, proteomic, and metabonomic technological advances over the past several years provide NTP with an unprecedented opportunity to evaluate the effects of chemical and physical agents at the most fundamental levels of biological organization. These new tools will form the basis of mechanism-based assays. The genomes of the species most frequently used by NTP - rats and mice- have been fully sequenced and cDNA and oligonucleotide microarrays are commercially available. Proteomics methodology is improving steadily. Genomic and proteomic capabilities will be essential to the NTP's future. There are a number of applications of these technologies, including the characterization of responses at fundamental levels of biological organization and the determination of mechanism of action based on gene expression profiles. It is now clear that virtually all toxic responses involve changes in gene expression that may be integral to the response (e.g., compounds that interact with ligand-dependent transcription factors such as steroid hormone receptors) or are in reaction to perturbations in cellular function. There are increasing numbers of papers in the literature describing mechanism-specific mRNA transcript profiles. As more information is generated, it is likely that transcript profiles will serve, among other methods, as a basis for mechanism-based testing. Genomics and proteomics will also provide essential data for improved biologically based dose-response modeling. Such modeling is a potentially powerful tool for understanding the nature of the dose-response curve at low levels of exposure, but is dependent on a firm understanding of the biological responses at a cellular and molecular level. Genomics and proteomics will energize biologically based dose-response modeling, which may become as routine in the future as pharmacokinetic modeling is today.

Although the "omics" technologies are powerful scientific tools, it is important to keep them in perspective. They can provide pattern recognition that correlates with toxicity, and these patterns may bring us closer to a mechanistic understanding; however, they do not directly provide mechanisms of toxicity.

Genomics technology will provide insight into the genetic sources of variability in toxicological response and aid in assessing risks among susceptible populations. The Environmental Genome Project will catalog the variations in gene sequence for key susceptibility

genes in a way that should define, at a molecular level, the characteristics of susceptible subpopulations. It should be possible to select (or create) animal models that are sensitive surrogates for these populations.

Metabonomics and non-invasive imaging are technologies that are now ready to be applied to toxicology studies. They are valuable for both determining the onset of and following the progression of diseases/toxicity. These methods have the potential to decrease the duration of studies, add value to the characterization of toxicity, and improve our understanding of the early stages of chronic diseases; such information may be valuable as the basis for hypotheses regarding prevention or treatment strategies in humans. Imaging technologies are developing that will allow multiple endpoints to be evaluated over time in the same animals, thereby enhancing the information that is obtained from a single animal and decreasing the number of animals needed.

Advances in genetics and developmental biology provide renewed hope that simpler (non-mammalian) animal models may have some utility in testing for potential human toxicity. Of particular interest are the observations that the genes that control processes of differentiation and pattern formation, cell proliferation and cell death are highly conserved across taxa, and that the number of different types of signal transduction pathways is limited. These findings suggest that effects on these key conserved pathways and regulatory points could be monitored in non-mammalian species whose biology has been well characterized, such as: *C. elegans*, Drosophila and zebrafish. While the results from testing in these species may not be definitive, they may be helpful in setting priorities for definitive testing. The relative ease of husbandry, fast generation times and short lifespan of these species, coupled with well-established techniques for experimental manipulation at the genetic, biochemical and cellular levels, should make it possible to screen orders of magnitude more compounds than can be accommodated using traditional mammalian models. While non-traditional models may be useful, the NTP should also consider human-derived models. It is critical, to the extent possible, to use human exposure conditions in developing new assays and models.

3. **How can we best structure the NTP to provide this information and to ensure its potential utilization in the protection of human health?**

The dissemination of the information generated by the NTP must be done in a way that ensures optimal utilization for public health protection. This effort must be viewed on two levels. The first level is to increase the interactions of the multiple agencies aligned with NTP. These communications must be continuous and open. Consequently, as the testing scheme evolves, the NTP needs to ensure that its multi-agency partners will use the data that are generated by any new or refined approaches, such as genomics, that are proposed and adopted. Currently, regulatory agencies (and ultimately the courts) use risk-based techniques to evaluate experimental information about effects from exposure to chemicals and make estimates of risk to human health and the environment. Regulators, the courts and, to a certain extent, the public, all understand the concept of reasonable or acceptable levels of risk. Clearly, since genomics will not be directly measuring an adverse effect, this approach will not be providing quantitative risk

information. The community (the regulatory agencies and the courts) that use such experimental data to regulate will need to be educated on the proper interpretation of the information being provided to enable its use, as appropriate, in the regulatory decision-making process. Furthermore, the general population must also be convinced that these are appropriate approaches, and will need to become educated about their utility and limitations
In carrying out its goal to provide information that can be used for making public health decisions, the NTP should consider providing information about these new techniques and findings from NTP studies to public health practitioners who provide support for toxicologists, chemists, pathologists, pharmacologists and biostatisticians at the state and local levels. The NTP could also help meet public health needs by expanding its efforts to produce and distribute information dealing with exposure estimates and dose response relationships.

The next level is to increase efforts in communication, education, and outreach; however, this is not a one-size-fits-all project. First, the NTP needs to identify the constituencies that they want to reach. Three groups seem to emerge: scientists who need to have easy access to data and reports; public health and medical professionals who need to implement what is known about what are and are not potential risks; and the general public who require appropriate material for personal decision making. The NTP has begun efforts aimed at basic scientists and these should be continued. However, the NTP must do a better job educating professionals such as risk assessors and risk managers who are responsible for making the decisions that directly impact public health. Working through professional organizations like the Society of Toxicology (SOT), the Society of Toxicology and Chemistry (SETAC) and the Society of Risk Analysis (SRA) will help convey the message to those who are using, or should be using, the information produced by the NTP.

Communication to the general public has the dual purposes of disseminating data and of increasing scientific literacy. These are not easy tasks. Scientists typically do a very poor job of reaching out to the public and communicating what they do to the average person. As science and technology continue to make advances, descriptions of what is being accomplished become more difficult for the public to understand and require use of carefully planned communication strategies.

To instigate effective communication to the general public, the NTP is encouraged to utilize the successful formats and networks of its partners. For example, NIEHS has a network of centers that all have community outreach and education programs. NIEHS and NCI have excellent web-based materials including activities and curricula for grades K-12. SOT currently conducts a K-12 program that is bringing the fundamental principles of toxicology to the classroom. A portion of the education message that the NTP needs to emphasize is what can and cannot be determined using animal models. Although the NTP has a goal to refine and replace animals in testing, this may be an unachievable goal. The general public needs to know the value of animal studies in protecting the public's health.

Overall, effective communication and education are essential as the NTP implements its vision in the 21st century.

4. **What resources will be needed to realize this vision and how long will it take?**

In order for the NTP to accomplish changes to develop a more mechanistic-based approach to hazard evaluation, a sizeable effort will be required. It seems doubtful that the NTP is likely to see sufficient staff growth to handle all of these activities internally. A close partnership with the extramural community should be considered. The human genome program might provide a useful model. The NTP could coordinate the effort, but extramural investigators would perform much of the discovery and development. Such an effort will be expensive and require several decades of work. If this is correct, then the NTP needs to first lay out the goals of such an effort, develop a strategy to achieve those goals, and, most importantly, have them reviewed and discussed in the scientific, stakeholder, and political communities prior to launching the effort.

Bringing this technology on-line and applying it appropriately will take not only an investment in equipment, but also in staffing. Bioinformatics, mass spectrometry, protein chemistry, and mathematical modeling will all be needed to supplement the excellent existing resources of the National Center for Toxicogenomics. It will also require a change in approach to testing. This "new" approach to testing will require an even more coordinated activity among disciplines, and must be more hypothesis-driven. Closer interactions with epidemiologists and those investigating exposure analysis will also be essential.

Support for training of future individuals capable of performing the research and being able to competently interpret the findings, including the biology, pathology, toxicokinetics, molecular biology, and informatics, will be essential. Also, because education and communication will be important for ensuring acceptance of the vision, the NTP should assure that individuals trained in risk and hazard communication are available.